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TROPHIC STATUS, OOGENIC STAGES, AND INSEMINATION OF RESTING POPULATIONS OF THREE *SIMULIUM* SPECIES (DIPTERA: SIMULIIDAE) IN CHIAPAS, MEXICO

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ABSTRACT. Of 176 black fly females collected resting at the edge of El Rosario River, 77% were caught in rocky shelters, whereas the others were captured on damp stretches of river bank. *Simulium ochraceum* predominated in the catches (74%), followed by *Simulium metallicum* (21%), and *Simulium callidum* (5%). Percentages of females without blood in their gut were 70, 85, and 67%, respectively. Of 55 *S. ochraceum* females that had recently oviposited (sacculate), 29 had the remains of a blood meal in the gut. Of 64 nulliparous females, 48 exhibited early oogenesis (Christophers's stage I–II) indicating recent emergence. At Las Golondrinas village, 36% (71) of 199 specimens were *S. ochraceum*, of these 85% had no blood in the midgut. Five females of *S. ochraceum* at this locality had red blood and 6 had black blood in their gut. Of 43 parous and 28 nulliparous females, 36 and 25 had ovaries at stages I–II, respectively. Most females of *S. metallicum* had no blood in their gut (92%). Nullipars were abundant (91) and stage N (24 h old) was observed in 66 of them.

Knowledge of the resting behavior of an insect vector is important in developing protocols to study host preference, species composition, and age structure (Service 1977).

After emergence teneral adults seek refugia near the breeding sites where they remain for a few hours. This time is essential for sclerotization of the integument and for final maturation of some organs and flight muscles, stomach epithelium, etc. Information on the natural resting sites of Simuliidae exist only as general accounts. Davies (1978) reported that teneral adults rest near a stream breeding site before dispersing. He also mentioned that blood fed females rest near their vertebrate hosts. Shemanchuk (1987) felt that the presence of gravid and blood-engorged females near streams indicates that they had returned to the river to rest after bloodfeeding.

In relation to onchocerciasis vectors in Central America, Dalmat (1955) assumed that the females rested in vegetation at night because they were attracted to the light of lanterns; however this phototaxis also could be an expression of host-seeking behavior. The same author reported that females of *Simulium ochraceum* Walker, *Simulium metallicum* Bellardi, and *Simulium callidum* Dyar and Shannon rested at different heights in trees in Guatemala. *Simulium ochraceum* was common flying over the canopy at 33 m.

Collection of simuliids in different types of natural microhabitats that exist near stream breeding sites or housing area host-seeking sites may detect sites suitable for control activities. Sampling during months when the population density is highest may increase the probability of finding the natural resting sites selected by each species. Finally, the capture of teneral males or uninseminated females may provide information about the behavior and postemergence biology of these simuliids, particularly *S. ochraceum*, which is the primary vector of onchocerciasis in southern Mexico and Guatemala.

In our paper we compare the trophic status and oogenesis development of females belonging to *S. ochraceum*, *S. metallicum*, and *S. callidum*, captured resting in natural sites located near a breeding site and in a village.

This study was conducted in a hyperendemic zone of onchocerciasis in southern Mexico. Simuliids were collected by sweep-netting at 2 localities: the El Rosario River (1,200 m above sea level) and Las Golondrinas village (850 m height), Acacoyagua Municipality, Chiapas, located at approximately 15°27'N, 92°40'W in the Sierra Madre of Chiapas (INEGI 1986). The distance between sites was about 8 km.

During January 15–19, 1992, the month in which simuliids reach peak numbers (Ortega and Oliver 1985), samples were taken at El Rosario River at 0900–1200 h and 1300–1600 h. Black flies were captured by sweeping over 4 muddy sites (about 2 × 1 m each) and 4 rock holes by making 20 sweeps with an aerial net evenly over each site. The rock hole site was 5 m^2 and located along the shaded shores of the river. Each rock hole included several sheltered sites among the rocks.

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Table 1. Trophic and gonotrophic status of female *Simulium ochraceum* and *Simulium metallicum* caught in natural resting sites at El Rosario River and Las Golondrinas village, Acacoyagua, Chiapas, Mexico.

Location	Species of *Simulium*	Trophic state[1]	Gonotrophic state[2]						
			Po	P(I–II)	P (III–V)	N	N(I–II)	N (III–V)	Total
El Rosario	*ochraceum*	NB	24	2	0	16	47	0	89
		RB	2	1	1	0	1	0	5
		BB	29	1	3	0	0	1	34
		Total	55	4	4	16	48	1	128
	metallicum	NB	14	0	0	8	6	0	28
		RB	0	0	0	0	2	0	2
		BB	0	0	1	0	0	2	3
		Total	14	0	0	8	6	0	33
Las Golondrinas	*ochraceum*	NB	2	30	4	3	21	0	60
		RB	0	2	0	0	3	0	5
		BB	0	4	1	0	1	0	6
		Total	2	36	5	3	25	0	71
	metallicum	NB	15	14	4	63	23	0	119
		RB	1	1	0	3	2	0	7
		BB	1	1	0	0	0	0	2
		Total	17	16	4	66	25	0	128

[1] NB—no blood, RB—red blood, BB—black blood.

[2] Po—parous just oviposited, P(I–II)—parous with primary follicle in resting stage (I–II), N—nulliparous with follicular stage at emergence, N(I–II)—nulliparous (without any remnants of sac dilatations) with follicles in resting stage, P(II–V) and N(III–V)—parous and nulliparous with terminal oogenesis stages in their follicles (Cupp and Collins 1979).

After sweeping, flies were killed with potassium cyanide and all specimens of *S. ochraceum, S. metallicum,* and *S. callidum* were dissected immediately to determine trophic status, parity, oogenesis stage (Cupp and Collins 1979), and the presence or absence of a spermatophore and spermatozoa in the spermatheca.

Flies capture were classified into 3 trophic stages: red blood, black blood, or no blood. Parity was determined by the presence of large-sac dilatations in the follicular tube, which is observed 2–4 h after oviposition (Cupp and Collins 1979). These sacs together with cellular debris become compacted and dilatations were evident 24 h after oviposition.

Nulliparous females were identified as having their follicles at any oogenesis stage but lacking saculate dilatations. The spermatophore, which is a hyaline spheroid object *ca.* 200 μm in diameter, was observed by squeezing gently the 8th sternite of the females. Spermathecae also were dissected to observe spermatozoa.

Resting black flies also were captured by sweeping at 10 sites in and around Las Golondrinas village. Around this community the terrain is very rugged. Sampling sites had an abundance of small crevices and hollows covered by weeds and faced either north or south, to avoid direct exposure to sunlight. Flies were collected from 50 samples. Two daily samplings were carried out during 25 days: 12 in February, 8 in March, and 5 in April. One sample was taken in the morning (0700–1000 h) and the other in the afternoon (1600–1900 h). Fifty sweeps were taken along 20 m at each sampling site and the black flies processed as above.

A total of 176 female simuliids was collected from the resting sites near the El Rosario River. Males were not collected. *Simulium ochraceum* was the most abundant species (74% of total) followed by *S. metallicum* (20%) and *S. callidum* (5%). Most (77%) flies were collected near rock holes; 82% of *S. ochraceum* and all *S. callidum* were collected in this microhabitat, whereas approximately equal numbers of *S. metallicum* were collected at rock holes (58%) and muddy sites (42%).

Although *S. ochraceum* was relatively abundant, the width of the El Rosario River (6–8 m) was not the optimum size for oviposition by this species. Dalmat (1955) reported that the typical breeding sites for *S. ochraceum* are small streams (width 0.3–1.7 m, depth 3–20 cm) that flow under the vegetation.

Of 161 dissected females (the rest had parasitic fungi in ovaries) caught at the river, 72% were unfed (Table 1). The percentage of unfed and fresh fed *S. ochraceum, S. metallicum* and *S.*

callidum were 70, 85, and 67% and 4, 2, and 2%, respectively. The number of recently oviposited *S. ochraceum* females (with large sacculate follicular tube) and with remains of black blood was 27%. One contained a relic ovum. Anderson (1987) reported that the presence of unlaid eggs (relic eggs) in the ovaries of *Simulium damnosum* Theobald females that have recently oviposited becomes increasingly common in females completing 2 or more gonotrophic cycles. Presence of black blood also can be an expression of advanced age because LeBerre (1966) observed that in *S. damnosum,* blood was digested more slowly in older than in younger females.

Of the 55 *S. ochraceum* females that recently had oviposited, 24 (44%) did not contain black blood, and 29 (53%) were positive for this characteristic. The highest numbers of females were parous recently oviposited (Po) and nulliparous with oogenesis in stage I–II. The percentages of these females from the whole catch were 43 and 38% for *S. ochraceum,* 42 and 24% for *S. metallicum* and 44 and 0% for *S. callidum.*

Collins et al. (1981) reported that parous *S. ochraceum* females bloodfed in the afternoon after ovipositing in the morning. They concluded that females returned from oviposition sites to the housing areas to refeed. We agree with Collins et al. (1981) that females must have a short resting time (less than 6 h) after oviposition.

Females at oogenesis stages N and I–II also were common in the catches (Table 1). These females were probably about 24 h old. Most of them had mated as evidenced by having sperm in the spermathecae and no spermatophore. Only 4 females had a spermatophore; two were captured at muddy sites at the river and 2 from rock holes. Five nulliparous, uninseminated females without a spermatophore also were collected in rock holes.

Of the 33 female *S. metallicum* captured, 14 had recently oviposited. Eight and 6 were nullipars in stages N and I–II, respectively. Only 2 had a spermatophore; one was collected at a muddy site and the other in a rock hole. There also were 2 uninseminated females without a spermatophore collected in rock holes. Only 9 *S. callidum* females were captured at the river; 4 had recently oviposited and 2 had red blood in the gut.

Table 1 shows the catches of *S. ochraceum* and *S. metallicum* resting sites at Las Golondrinas village. No *S. callidum* females were caught. All of the 199 black flies captured were females; 71 were *S. ochraceum* and 128 were *S. metallicum.* For *S. ochraceum* 60 females appeared unfed, 5 had red blood remains in the gut, and 6 had black blood in the gut. Of the females with no blood, 36 were parous and 24 nulliparous. Only 2 had recently oviposited, and a high proportion (50%) had oogenesis at stage I–II. This suggests they were resting after arrival from the breeding site. Only 4 had oogenesis in the last stages (III–IV) and these females were also scarce in the resting sites close to the river.

Of the 24 nullipars with no blood, 3 were in stage N (24 h old) and 21 were in stage N(I–II). Fifty-five females (75%) had a spermatophore whereas only 2 were uninseminated. Neither of the latter had a spermatophore, which means that they arrived at the dwelling area without mating.

Finally, the total catch of *S. metallicum* (128), was almost twice that of *S. ochraceum.* One hundred and nineteen females had no blood in the digestive tract (93%), 7 had red blood remains, and just 2 had black blood vestiges. Of 128 females, 37 (29%) were parous and 91 (71%) nulliparous. Of the parous flies 15 had recently oviposited and 14 were in the initial stages of oogenesis (I–II). Only 4 parous females showed advanced oogenesis (III–IV). Of the 91 nulliparous flies, 73% were in stage N and 27% were in I–II. With regard to reproductive status, 112 (94%) of the nonbloodfed females had a spermatophore.

In general, the incidence of females with remains of red blood in the gut was very low in the catches. No blood-engorged black flies were captured during this study. All females that had bloodfed had only small remains of the blood (red or black blood). The lack of males and blood-engorged females strongly suggests that the sampling included biased portions of the population. It is possible that blood-engorged females have a very short flight after taking the blood meal. Their first resting sites may be shaded sites near dwellings (walls, roofs, etc.). These sites were not included in this research because they are not considered as natural resting sites. However, bloodfed females may be widely dispersed in and around the village, which together with a very rugged terrain would make it very difficult to obtain a large number of flies unless many collectors are employed.

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